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QSAR and docking studies on inhibition of carbonic anhydrase tumor-associated isoenzyme IX with heterocyclic sulfonamides as cytotoxic agents

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ABSTRACT

Carbonic anhydrases (CAs) are metalloenzymes containing one zinc ion (Zn^{2+}) per polypeptide chain. Sulfonamide derivatives incorporating substituted triazine ring have been investigated as excellent cytotoxic agents that inhibiting carbonic anhydrase tumor-associated isoenzyme IX.QSAR and docking studies for45 sulfonamide derivatives were performed. Twenty two QSAR models were constructed each model contain eight equations. The best of these models was chosen based on its statistical validation parameters where the R^2 value was 0.98.Top model was returned based on six molecular descriptors; two fast descriptors, two spatial descriptors and two VAMP electrostatics descriptors. External validation of the developed model was governed by calculating the predicted biological activity and the residual values for training and test sets. These calculated values revealed a high prediction ability of our developed model. Molecular docking study aims to interpret the comparative differences in the binding interactions of these compounds at molecular level as inhibitors of CA IX.The sulfonamide ($-SO_2NH_2$) group of all docked compounds form hydrogen bond with Thr199 and is coordinated to zinc ion in the active site. Docking study revealed that the potency of the inhibitors was reduced by the presence of either bulky alkyl, orphenoxy substituent on the triazine ring. The potency was markedly enhanced by the presence of free aminoor amino acid substituents on triazine ring.

Keywords: carbonic anhydrase enzyme, antitumor, inhibitors, sulfonamide, triazine, QSAR, genetic function approximation, docking.

INTRODUCTION

Carbonic anhydrases (CAs) are metalloenzymes [1] containing one zinc ion (Zn^{2+}) per polypeptide chain, whose main physiological function is to catalyze the reversible hydration of carbon dioxide to bicarbonate anion and proton ($CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$) [2, 3]. The metal ion is critical for catalysis, as the apoenzyme is devoid of any catalytic activity [4].

The chemical species involved in the CA-catalyzed processes, CO_2, bicarbonate, and protons, are essential molecules/ions in many physiologic processes in all organisms. Thus, CAs play crucial roles in processes connected with respiration and transport of CO_2/bicarbonate, pH and CO_2 homeostasis, electrolyte secretion in a variety of tissues/organs, biosynthetic reactions (such as gluconeogenesis, lipogenesis and ureagenesis), bone resorption, calcification, tumorigenicity, and many other physiologic or pathologic processes [3, 4]. These enzymes are ubiquitous in all kingdoms such as Archaea, Bacteria, algae, green plants as well as superior animals including vertebrates, and are encoded by five distinct, evolutionarily unrelated gene families: the α-CAs (present in

vertebrates), the β-CAs (mainly present in Bacteria and plants), the γ-CAs (mainly in Archaea), and the recently isolated δ- and ε-classes of CAs (present in marine diatoms and chemolithoautotrophic bacteria, respectively)[5].

Sixteen human CA isoenzymes (hCA) belonging to alpha class have been identified. Twelve of them are characterized by a common Zn(II) ion at the catalytic site, while they differ in organ and tissue expression, cellular localization, molecular features, catalytic activity and responsivity to different classes of inhibitors [1].There are five cytosolic forms (CA I, CA II, CAIII, CA VII, and CA XIII), five membrane-bound forms (CAIV, CAIX, CA XII, CA XIV, and CA XV), two mitochondrial forms (CA V_A and CA V_B), whereas CA VI is secreted in saliva and milk [3, 6]. Recent evidence also implicates other CA isoenzymes in progressive kidney disease and in idiopathic chronic pancreatitis [7].

CAIX is a special member of CA family. It is confined to a limited number of normal tissues, but highly over expressed in many solid tumors types, such as gliomas, mesotheliomas, papillary carcinomas, follicular carcinomas, carcinomas of the bladder, uterine cervix carcinomas, nasopharyngeal carcinomas, head and neck cancer, mastocarcinoma, esophagus cancer, lungs and brain cancer, squamous/basal cell carcinomas, and kidney tumors, among others. Moreover, it is involved in important processes connecting with tumor growth. The expression of CA IX is regulated by hypoxia inducible factor 1 (HIF-1)cascade, strongly unregulated by hypoxia and down regulated by the wild-type von Hippel–lindau tumor suppressor protein (pVHL) [6].Among CA isoenzymes, the transmembrane proteins CA IX and XII have recently emerged as promising therapeutic targets for the treatment of human cancer.

CO_2 is the main byproduct of all oxidative processes and is produced in large amount in metabolic active tissues such as tumors, where CA IX and XII are highly over expressed in response to hypoxia inducible factor (HIF)pathway. Since the spontaneous hydration of CO_2 is a very slow process, the catalytic activity of CAs assumes a pivotal role in the maintenance of CO_2/HCO_3^- homeostasis. In hypoxictumor cells, both isoforms contribute to extracellular acidification and to maintain intracellular pH more alkaline; thus, promoting tumor cell survival in an acidic environment and low bicarbonate medium. Therefore, considering that in normal tissues the expression levels of CA IX and XII are extremely low, the pharmacological targeting of these tumor-associated isozymes is increasingly asserting as a valuable approach in the search for novel anticancer treatments with reduced or absent side effects [1].

The crystal structure of the catalytic domain of the tumor-associated human carbonic anhydrase IX was reported in 2009.The purified hCA IX is a dimeric protein, and its dimerization is mediated by an intermolecular disulfide bond involving Cys-41.The CA IX active site is located in a large conical cavity, which spans from the surface to the center of the protein. The zinc ion is located at the bottom of this cavity. Two distinct regions made of hydrophobic or hydrophilic amino acids delimit the active site [8].

Careful examination of the existing literature seems to attribute a different role to each of the CA IX domains. The main player in the growth and survival of tumor cells seems to be the CA IX catalytic domain that convertsCO_2, produced in the cytoplasm of hypoxic cells and diffused through the plasma membrane, into bicarbonate and proton contributing to extra-cellular acidosis. The newly generatedHCO_3^-ions could then be transported back into the tumor cells or to blood capillaries by HCO_3^- transport proteins. The coupled transport process is probably essential for hypoxic cancer cells to buffer their intracellular pH value to near neutral conditions necessary for their biosynthetic reactions [9].

CAIX is implicated in cell adhesion as well as in acid–base balancing and intracellular communication. High tumoral carbonic anhydrase IX (CA IX) expression is associated with poor prognosis, tumor progression and aggressiveness.CA IX was identified as a potentially important marker of hypoxia. Furthermore, CA IX overexpression is often associated with a poor responsiveness to the classical radio- and chemo-therapies. Therapeutic inhibition of CA IX resulted in decrease primary tumor growth and metastasis in preclinical breast tumor models. From a therapeutic point of view, acidic pH is also related to a chemoresistance by a decrease in uptake of weakly basic anticancer drugs as irinotecan, mitomycin, bleomycin and doxorubicin[3].

Due to their important role, inhibition of these enzymes by carbonic anhydrase inhibitors (CAIs) was a target for the design of therapeutic agents useful in the treatment and prevention of many diseases [5].α-CAsare inhibited by several main classes of inhibitors: inorganic anions, sulfonamides and their isosteres (sulfamates and sulfamides),

phenols,[10] coumarins[11, 12] and antibodies [13].Most of CA IX inhibitors have a sulfonamide or sulfamate moiety able to coordinate the zinc ion of catalytic binding site and inhibit the enzymatic activity [6, 12].Although inhibition of CAs by aromatic/heterocyclic sulfonamides has been clinically exploited for more than 45years in the treatment of a variety of diseases such as glaucoma, epilepsy, congestive heart failure, mountain sickness, gastric and duodenal ulcers, or as diuretic agents, their potential use as antitumor drugs has little been explored up to now. Several classical clinical agents from this class include acetazolamide, methazolamide orethoxzolamide [14].With the sulfanilamides as the lead structure, different classes of pharmacological agents have been obtained such as antibacterial, hypoglycemic, antithyroid drugs, and others [5]. However, there are a limited number of compounds that exhibit high selectivity toward CA IX[1].

Discovering new CA IX inhibitors become a good target to design new-type anticancer drug [15, 16], which encourage us to start this research to study the relationship between chemical structure and biological activity. Quantitative structure–activity relationship (QSAR), is a method for building computational or mathematical models which attempts to find a statistically significant correlation between structure and function using a chemometric technique. In terms of drug design, structure here refers to the properties or descriptors of the molecules, their substituents or interaction energy fields, function corresponds to an experimental biological/biochemical endpoint like binding affinity, activity, toxicity or rate constants, while chemometric method include MLR, PLS, PCA, PCR, ANN, GA etc [17]. Quantitative structure–activity relationship (QSAR) study is a tool of prediction endpoint of interest on organic compounds acting as drugs, which have not been experimentally determined. Many physiological activities of compound scan be related to their composition and structure. Since topological indices are the numerical representation of molecular structure, they are the best candidates for QSAR studies[14].

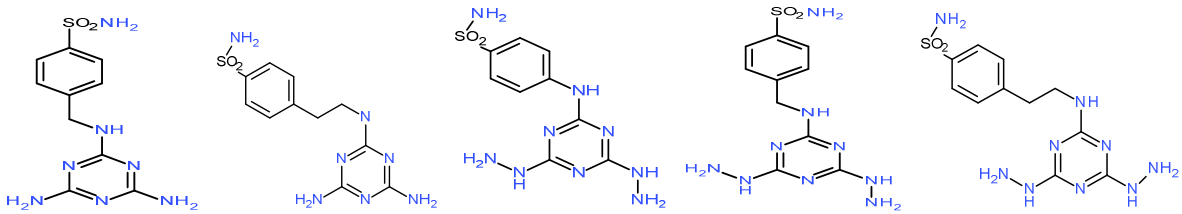
The inhibition of CAIX isoenzyme with a series of triazinyl sulfonamides and six clinically used derivatives have been investigated [18]. Indisulam (E7070) IND, an antitumour sulfonamide in phase II clinical trials for which they recently demonstrated potent CA inhibitory properties, has also been included in this study[18, 19]. A very interesting inhibition profile against CA IX with these sulfonamides had been observed. Several nanomolar (K_I ranged from1–640nM) CA IX inhibitors have been detected, among the triazinylsulfonamides examined[18].

Since CA IX is a highly active isoenzyme predominantly expressed in tumor tissues with poor prognosis of disease progression, QSAR and docking studies on such an interesting class of inhibitors of triazinyl sulfonamides is yet to be performed; we have undertaken the present study, which will be very promising for the potential design of CA IX specific inhibitors with application as anti-tumor agents. Molecular docking study aims to interpret the comparative differences in the binding interactions of these compounds at molecular level as inhibitors of CAIX.

MATERIALS AND METHODS

QSAR

Biological activity data: The structure of triazinyl sulfonamides together with the six clinically used inhibitors: acetazolamide (39), brinzolamide(40), dichlorophenamide(41), dorzolamide(42), ethoxzolamide(43), indisulam (E7070) (44)and methazolamide (45)are shown in Figure 1. Our data set comprised of 45 compounds. The training set has 30 compounds while the remaining 15 compounds constitute the test set.



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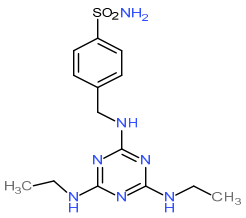

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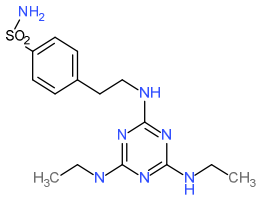

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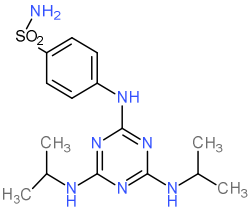

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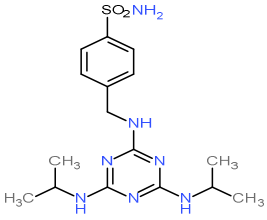

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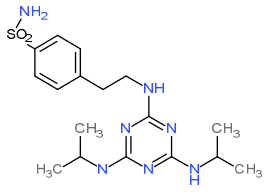

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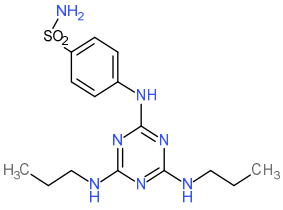

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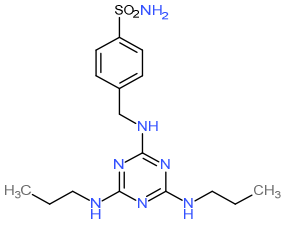

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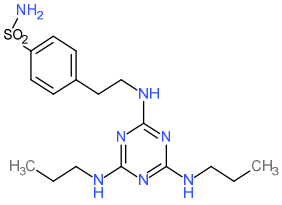

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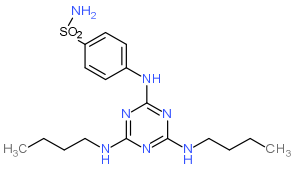

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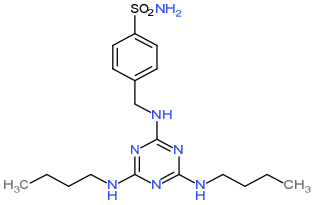

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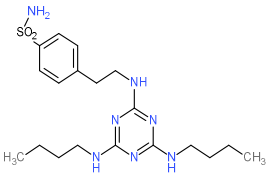

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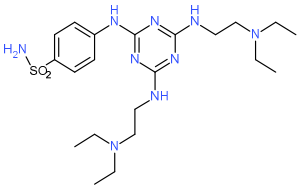

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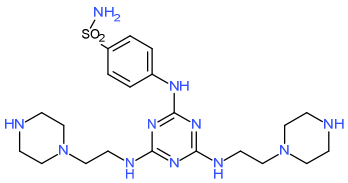

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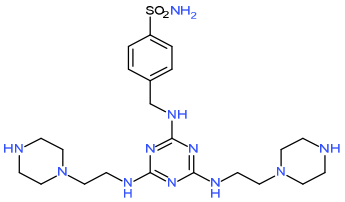

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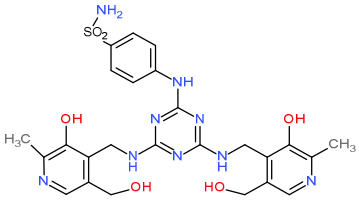

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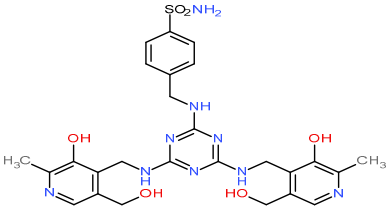

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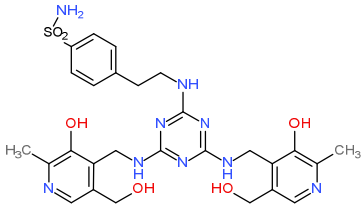

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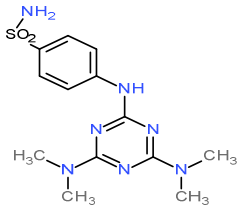

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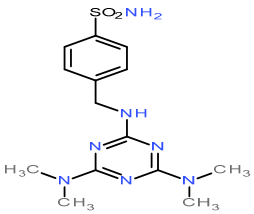
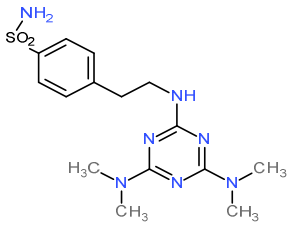
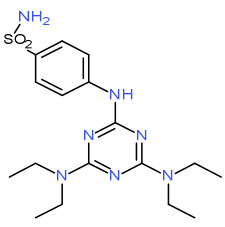
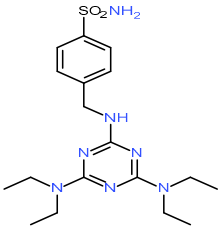








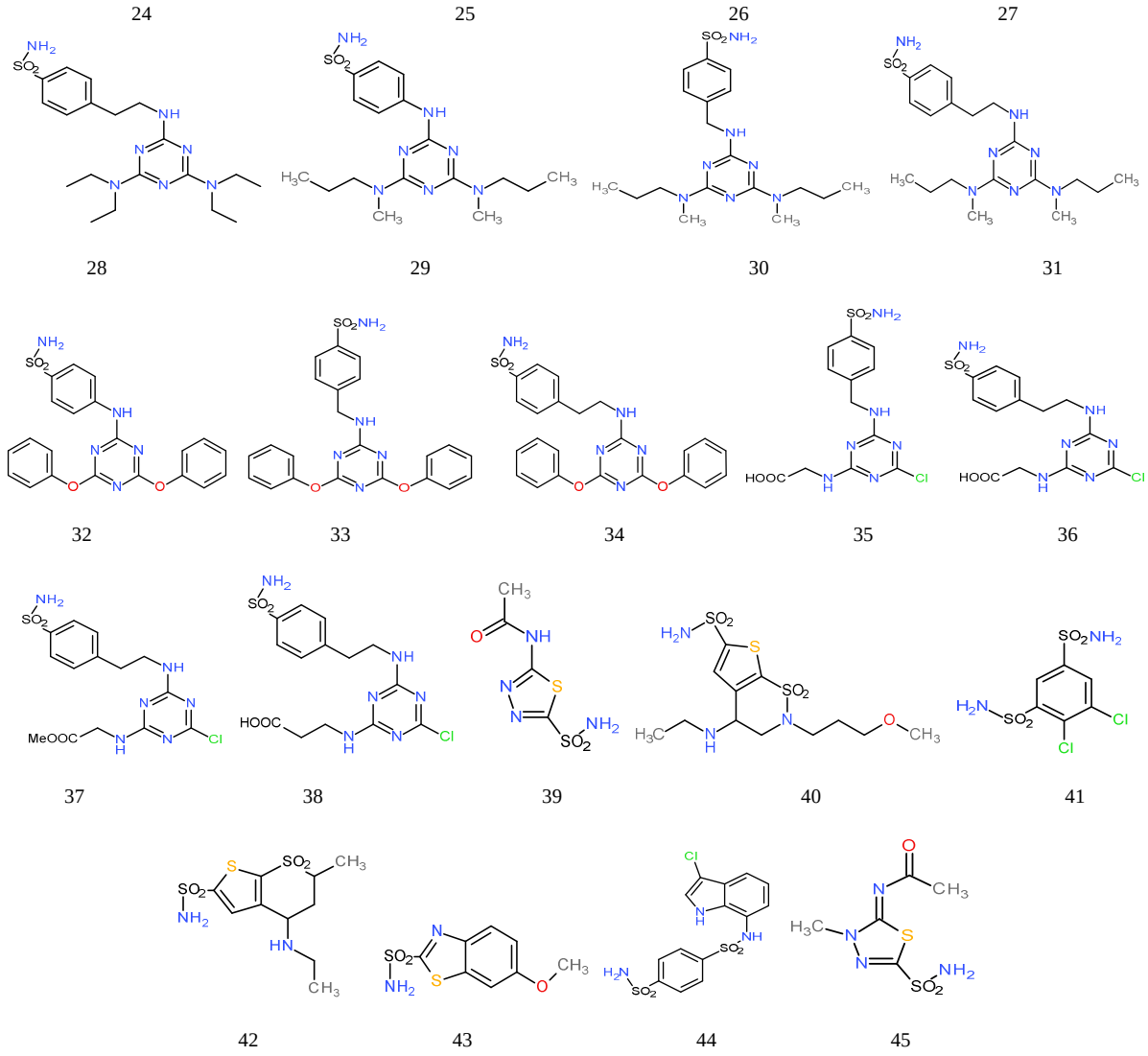


Figure 1. Chemical structures of sulfonamide derivatives used in our dataset

The set of sulfonamides and their hCAIX experimental K_I values were obtained from literature [18] and are listed in Tables 1 and 2. These activity values were converted into their log $(1/K_I)$,and used as dependent variable for modeling. The training set was used to build the model using Accelrys® Materials Studio (MS5.0) software [20]. The test set was used to evaluate its prediction ability.

Geometry optimization: 3D Structures were drawn and geometry optimized using the ChemAxon® MarvinSketch 5.1.4 [21]. These structures were further geometry optimized using the Vienna ab-initio Molecular dynamics Package (VAMP) module [22]. Different algorithms were used to give the best output structure energy. The consensus flexible alignment was done in relation to X axis using the root mean square (RMS) with field fit method by employing a combination of steric and electrostatic field.

Table1. Biological activity data and external validation of the developed model by calculating the residual values for training set using equation (1)

Comp. No.	K_I (nM)	log (1/ K_InM) (practical)	Log (1/K_InM) (predicted)	residual value	Comp. No.	K_I (nM)	log (1/ K_InM) (practical)	log (1/ K_InM) (predicted)	residual value
6	2.5	-0.91629073	-1.20983	-0.29354	**27**	7.5	-2.01490302	-1.93864	0.076262
7	2.9	-1.06471074	-1.40331	-0.3386	**28**	6.3	-1.84054963	-1.7545	0.086048
8	13	-2.56494936	-2.31935	0.245598	**29**	10.5	-2.35137526	-2.44821	-0.09683
9	16	-2.77258872	-2.8105	-0.03791	**30**	12.6	-2.53369681	-2.11606	0.417638
10	18	-2.89037176	-2.88782	0.002548	**31**	13.7	-2.61739583	-2.62388	-0.00649
11	56	-4.02535169	-4.10022	-0.07487	**33**	197	-5.28320373	-5.36511	-0.08191
12	78	-4.35670883	-4.11377	0.242942	**34**	113	-4.72738782	-5.31287	-0.58549
13	125	-4.82831374	-4.86229	-0.03397	**35**	1.7	-0.53062825	-0.47964	0.05099
14	185	-5.22035583	-4.8812	0.339151	**36**	1	0	-0.26137	-0.26137
15	210	-5.34710753	-5.20947	0.137635	**39**	25	-3.21887582	-3.07274	0.14614
18	287	-5.65948222	-6.23692	-0.57744	**40**	37	-3.61091791	-3.63401	-0.02309
19	274	-5.61312811	-4.80737	0.805763	**41**	50	-3.91202301	-4.46685	-0.55483
21	423	-6.04737218	-6.0811	-0.03373	**42**	52	-3.95124372	-3.81688	0.134364
22	640	-6.46146818	-6.65804	-0.19657	**44**	24	-3.17805383	-3.27226	-0.09421
26	5.9	-1.77495235	-1.74931	0.025643	**45**	27	-3.29583687	-2.81385	0.481984

Table2. Biological activity data and external validation of the developed model by calculating the residual values for test set using equation (1)

Comp. No.	K_I (nM)	log (1/K_InM) (practical)	Log(1/K_InM) (predicted)	residual value	Comp. No.	K_I (nM)	log (1/K_InM) (practical)	log(1/K_InM) (predicted)	residual value
1	1.2	-0.18232156	-0.05039	0.131933	**37**	1.4	-0.33647224	-0.42311	-0.08664
2	1	0	-0.09154	-0.09154	**38**	1.2	-0.18232156	-0.21693	-0.03461
3	1.5	-0.40546511	-0.95051	-0.54504	**17**	174	-5.1590553	-5.45479	-0.29573
4	1.4	-0.33647224	-0.30651	0.029966	**16**	204	-5.31811999	-5.40476	-0.08664
5	1.3	-0.26236426	-0.01753	0.244831	**20**	386	-5.95583737	-6.03859	-0.08276
23	1.5	-0.40546511	-0.7797	-0.37424	**32**	254	-5.53733427	-5.36728	0.170058
24	1.3	-0.26236426	0.433776	0.69614	**43**	34	-3.52636052	-2.79991	0.726452
25	1.5	-0.40546511	-0.3245	0.080963					

Building the QSAR model: Genetic function approximation (GFA), a statistical modeling algorithm, was used to build the model using the most simplest fast descriptors which either one dimensional (1D) or two dimensional (2D) and the most complex three dimensional (3D) atomistic descriptors, VAMP electrostatics, spatial descriptors and forcite energetics [23].

Validating the model: All constructed models were validated using the reported validation parameters[24]. These parameters include Friedman lack of fit (LOF), the squared correlation coefficient (R^2), adjusted R^2, cross validated R^2 (CV) and significance of regression (SOR) F-values of the training set in addition to R^2 value of the test set [24, 25]. Scaled LOF smoothness parameter was set to default of 0.5.

Docking

Docking process needs a three dimensional (3D) structure of both inhibitors and isoenzyme (hCAIX). 3D structures of docked compounds were drawn and geometry optimized into ChemAxon® MarvinSketch 5.1.4 [21]. Library of studied compounds was generated using Mona software [26].CA IX crystal structure is available in protein data bank, (ID: 3IAI)[27].All of these sulfonamide derivatives were docked into the hCA IX active site using FlexX module in LeadIT 2.1.8 software-package [28]. FlexX flexibly places ligands into the active site with an incremental buildup algorithm (pose clustering) [29]. It starts with selecting a base fragment, which is placed into the active site based on superposing interaction points of the fragment and the active site (pattern recognition technique). The base fragment is then incrementally built up to the complete compound by modeling the ligand flexibility with a torsion library for the added components [30]. Placement of the ligand is scored based on protein–ligand interactions where, the binding energy for solutions generated is estimated, and placements are ranked [31].

The Lead IT suite provides the FlexX-scoring function, which was used to find the initial best poses [32]. For final evaluation of the poses of the ligands affinity toward docked enzyme, the scoring function (Hyde) was used. For scoring analysis; the best FlexX and HYDE score for each compound was taken and compared to the scores of the other compounds.

RESULTS AND DISCUSSION

QSAR

Genetic function approximation (GFA) was employed to search for the best possible QSAR regression equation capable of correlating the variations in biological activities of the training compounds with variations in the generated descriptors (multiple linear regression modeling (MLR)). GFA method was used to carry out both data reduction and parametric regression simultaneously. The equation length was set to make number of variables do not exceed one third to one fifth the number of data. The quality of the model was improved by involving three dimensional (3D) shape descriptors which revealed that stereochemichal parameters have remarkable effect on biological activity.

Twenty two models were constructed each model contain eight equations. Top model was returned based on six molecular descriptors. The best developed equation (1) under consideration is found as below:

$$\begin{aligned}\text{Log } (1/K_I) = {} & 45.374689489 * X55 \\ & + 14.573335929 * X62 \\ & - 0.036221396 * X127 \\ & + 0.000066195 * X171 \\ & + 0.561872486 * \text{ramp}(X19 - 37.053056786) \\ & + 31.998327054 * \text{ramp}(- 0.310921912 - X59) \\ & + 9.492828822\end{aligned}$$

The involved molecular descriptors in the developed equation (1) are defined as following:
X55: BK: N1(3) : Coulson charge (VAMP Electrostatic descriptors)
X62: BU: Molecular density (Spatial descriptors)
X127: (S: Kappa-1 (alpha modified) (Fast descriptors))^2
X171: (BS: Molecular area (vdW area) (Spatial descriptors))^2
X19: V: Subgraph counts (0): path (Fast descriptors)
X59: BQ: N2(2) : Mulliken charge (VAMP Electrostatic descriptors)

The generated model was based on six descriptors; two fast descriptors, two spatial descriptors and two VAMP electrostatics descriptors. Fast descriptors are topological indices which based on graph theory concepts [33]. Topological indices are 2D descriptors which take into account the internal atomic arrangement of compounds and encode in numerical form information about molecular size, shape, branching, presence of heteroatoms, and multiple bonds[34].The positive co-efficient of spatial descriptors indicate that the size and shape of molecule play important role in inhibiting hCAIX isoenzyme. VAMP Electrostatics descriptors predict geometries, heats of formation, and a host of molecular properties, including ionization potential, multipole moments, molecular and atomic polarizabilities, and potential-derived charges [35]. They provide a means of estimating partial atomic charges calculated by the methods of computational chemistry, particularly those based on the linear combination of atomic orbitals molecular orbital method. The large value of coefficient of electrostatic descriptors revealed that the presence of aminogroup on sulfonamides ($-SO_2NH_2$) and another amino group in the compound is the most effective parameter and is favored for inhibiting the hCAIV enzyme.

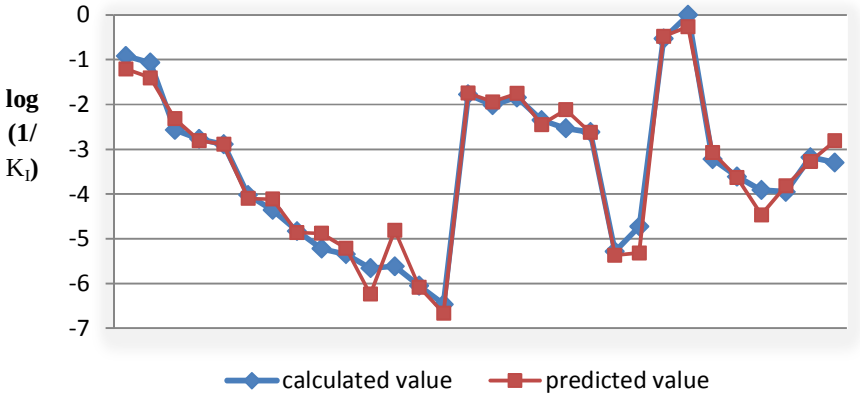
The internal validation parameters calculated for the model represented by equation (1) are shown in Table 3 where the R^2 value was found to be acceptable (0.98). The difference between the R^2 and adjusted R^2 value is 0.003311 which indicates that the number of descriptors involved in the QSAR model is acceptable. The number of descriptors is not acceptable if the difference is more than 0.3 [24]. A common method for internally validating a QSAR model is cross-validation. A cross-validated R^2 is smaller than the overall R^2 for equation(1). It is used as adiagnostic tool to evaluate the predictive power of anequation. The internal validation [36] results prove that the developed QSAR model represented by equation (1) is accepted in terms of good correlation coefficient and low LOF value.

Table 3. The internal validation parameters calculated for the developed QSAR model

Internal validation parameters	Equation 1
Friedman LOF	0.38460700
R-squared	0.98123800
Adjusted R-squared	0.97792700
Cross validated R-squared	0.97349200
Significant Regression	Yes

The only way to estimate the true predictive power of a QSAR model is to compare the predicted and observed activities of an (sufficiently large) external test set of compounds that were not used in the model development[24].External validation [36, 37] of the developed model is achieved by calculating the predicted biological activity for test set using equation (1). These calculated values revealed a good prediction ability of our developed model as shown in Figure 2 (a & b).

(a) External validation of training set



(b) External validation of test set

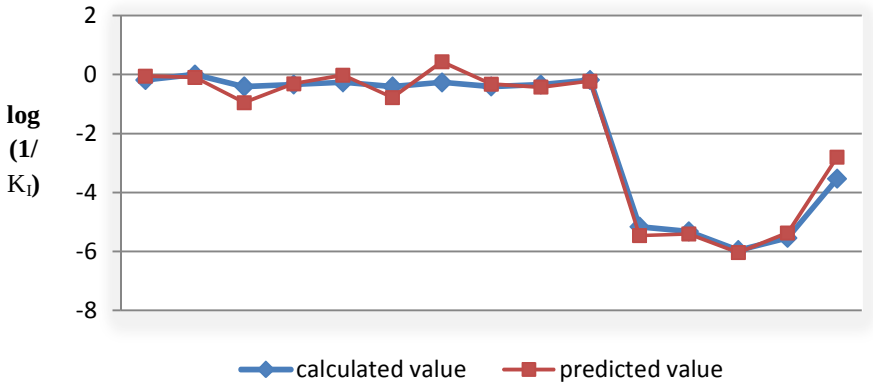


Figure 2. External validation of the developed model, the plot of predicted log ($1/K_I$) versus experimental values for training set (a), and test set (b)

The residual values are calculated from the difference between the actual and predicted biological activity values ($\log 1/K_I$) for training set as shown in Table 1and test set as shown in Table 2.The developed model revealed very good predictability as shown in Figure 2 (a &b) and Tables (1 & 2) where residual values ranged from -0.00649 to -0.58549.

Docking

To understand the mechanism of action of CA IX inhibitors, we have to consider first the mechanism of action of CA IX enzyme and the catalytic role of the zinc ion.The active form of the enzyme is the basic one, with hydroxide

bound to Zn^{2+}. The basic active form of the enzyme is a strong nucleophile that attacks CO_2 molecule leading to the formation of bicarbonate coordinated to Zn^{2+}. The bicarbonate ion is then displaced by a water molecule and liberated into solution, leading to the formation of the inactive acid form of the enzyme, with water coordinated to Zn^{2+}[38] as shown in the following equations:

$$E\text{-}Zn^{2+}\text{-}^{-}OH + CO_2 \leftrightarrow E\text{-}Zn^{2+}\text{-}HCO_3^{-}$$
$$E\text{-}Zn^{2+}\text{-}HCO_3^{-} + H_2O \leftrightarrow HCO_3^{-} + E\text{-}Zn^{2+}\text{-}OH_2$$

The unsubstituted sulfonamides inhibit CAs by binding to the Zn^{2+} ion of the enzyme by nucleophilic substitution of the non-protein zinc ligand [38] as shown in the following equation:

$$E\text{-}Zn^{2+}\text{-}^{-}OH + Ph\text{-}SO_2NH_2 \leftrightarrow E\text{-}Zn^{2+}\text{-}^{-}NHSO_2Ph + H_2O$$

The metal ion (which is a Zn^{2+} ion in all α-CAs investigated up to now) is essential for catalysis.

X-ray crystallographic data show that, the CA IX active site is located in a large conical cavity, which spans from the surface to the center of the protein. The zinc ion is located at the bottom of this cavity. Two distinct regions made of hydrophobic and hydrophilic amino acids delimit the active site. In particular, Leu91,Val121, Val131, Leu135, Leu141, Val143, Leu198, and Pro202define the hydrophobic region, while Asn62, His64, Ser65, Gln67, Thr69, and Gln92 identify the hydrophilic one[8, 38].

We have performed docking studies for compounds (1–45) within the active site of hCA IX, by using hCA IX crystal structure taken from Protein Data Bank (PDBcode : 3IAI) [27].Removal of water molecule from the active site eliminate the zinc ion from the working platform which revealed that these water molecules are essentially involved and bound to the active site for catalytic function. Some of these docked sulfonamides bind to conserved water molecule in the active site.

The predominantly common attraction forces observed in this study between the active site of the enzyme and docked inhibitors are of two main types; firstly, formation of hydrogen bonds (H-bonds) with two main amino acids Thr199 (one of the gate keeper residues of this enzyme[14])and, Gln67 as well as two conserved water molecules (water1412 and water1489); secondly, hydrophobic van der Waals contacts with Val121, Leu198 and His94.

The sulfonamide($-SO_2NH_2$) function group of all docked compounds form H-bond with Thr199. The nitrogen of the sulfonamide group of all docked compounds is coordinated to the zinc ion which is considered the essential feature for activity. It was observed that, the most active compounds have H-bonds between Gln67andaminotriazine ring. Bulky substituents on the aminotriazine ring block this interaction and reduce the activity. All docked compounds have a hydrophobic attraction force with His94.Formation of H-bond with water molecules (water1412 and water1489)enhanced the potency of the inhibitors.

We concentrated our discussion on some derivatives, the strong and weak inhibitors. Compounds (1- 22) have two amino or monosubstituted amino groups on triazine ring as shown in Figure 1.The sulfonamide group ($-SO_2NH_2$) is the key for inhibiting the CA IX enzyme. The formation of H-bond between Thr199 is observed for all docked compounds which helped the nitrogen atom to coordinate to the Zn^{2+} ion located in the active site as shown in Figure 3. The most active inhibitor in this group, compound (2), showed that the aminotriazine ring played an important role because it formed two H-bonds with Gln67 and the free amino group formed another hydrogen bond with water1489 which contribute to the K_I value of 1.0 nM. The oxygen of sulfonamide group formed H-bonds with Thr199 and Thr200. Stereochemicaly, The 3D structure of this molecule in active site is ideal for making hydrophobic attraction force with Gln67, Thr200 and His94.

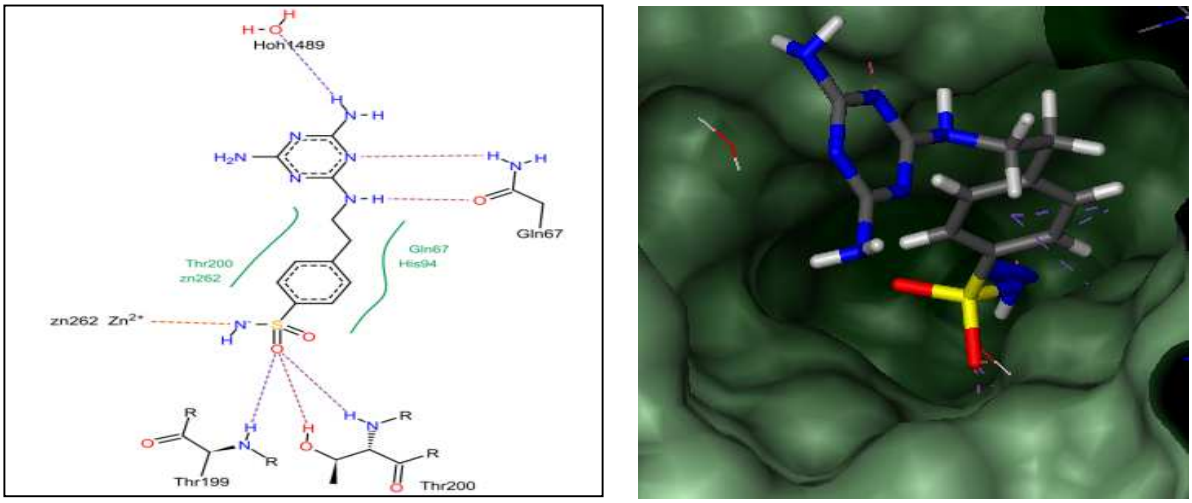


Figure 3.2D and 3D pose views of compound (2) docked on the active site of hCA IX

In contrast, the least active compound in this group, compound (22, K_I=640nM), the bulky side chain on triazine ring, prohibited the H-bond interaction between Gln67 and aminotriazinering as shown in Figure 4. The bulky aromatic side chain makes the molecule bind to amino acid residues located outside the active site and does not probably impeded on it, which dramatically affect the inhibitory effect of this compound.

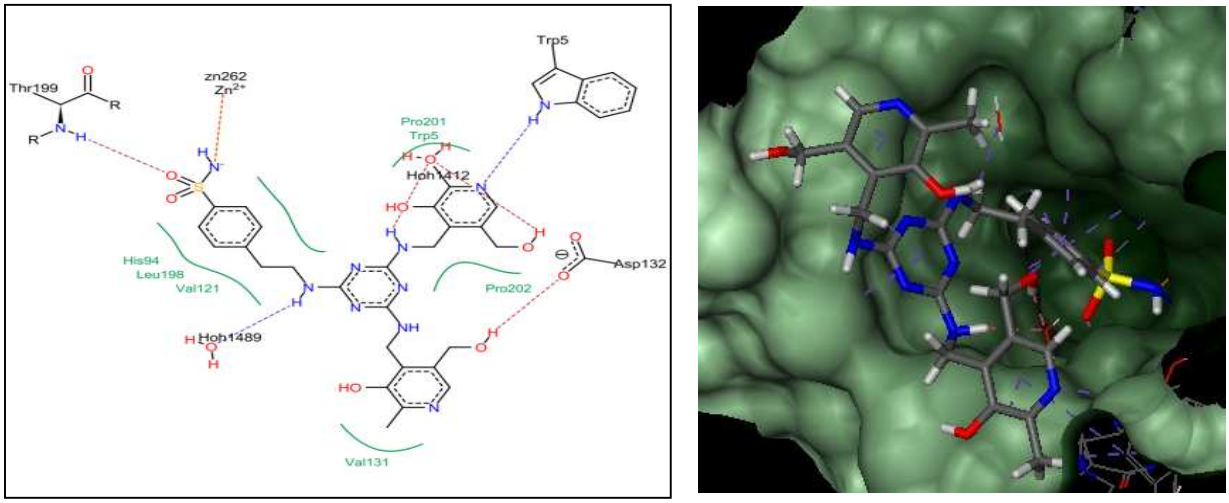


Figure 4.2D and 3D pose views of compound 22 docked on the active site of hCA IX

On conclusion sulfonamide group is essential for activity. Inhibitors bearing free NH_2 or RNH group on triazine ring are potent inhibitors while the presence of bulky alkylamino groups on triazines such as compounds (15-22) reduces the activity as evidenced in our developed QSAR model. Bulky side chain on triazine block its binding to Gln67 which dramatically reduce potency while, free amino group on triazine ring enhance potency by interacting with water molecules. The presence of hydrazine group on triazine ring enhance the activity as in compound (3, K_I = 1.5nM). The hydrazine group formed H-bonds with water1412, water1489 and Gln67. The small size of hydrazine group makes it possible to aminotriazinering to form H-bond with Gln67 as shown in Figure 5.

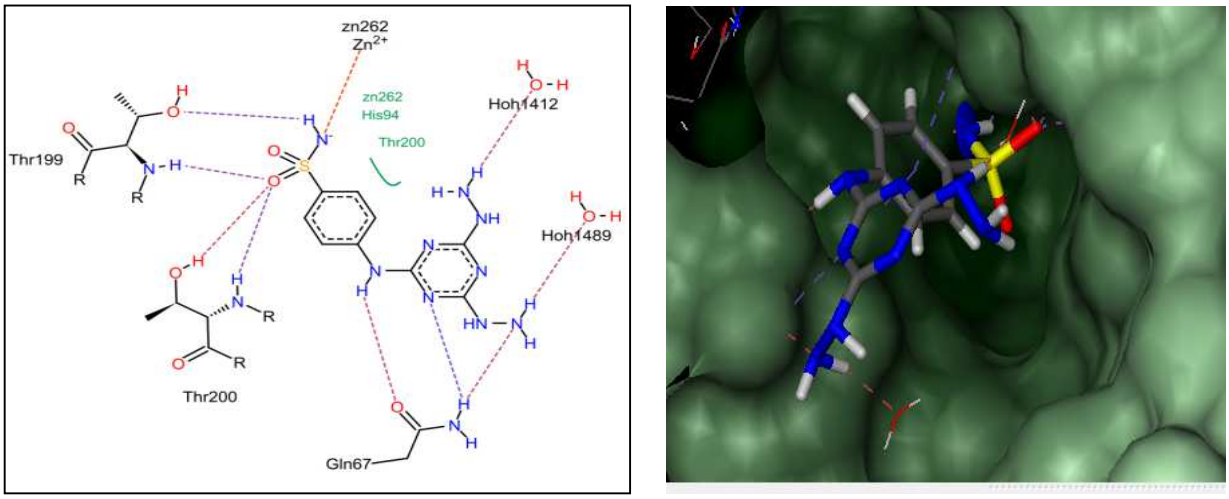


Figure 5.2D and 3D pose views of compound 3 docked on the active site of hCA IX

Regarding to inhibitors (23-31) having disubstituted amino group on triazine ring as shown in Figure (1),there is noH-bond to water molecules was observed while they all formed H-bond to Thr199. Compound (24), the most active compound (K_I=1.3 nM),the sulfonamide group formed H-bond with Thr199 and Gln67 while the nitrogen coordinated to Zn^{2+}ion. The aminotriazine ring formed H-bonds with Gln67. Van der Waals interactions were observed with Gln67, Val121 Leu198 and Thr200 as shown in Figure 6.

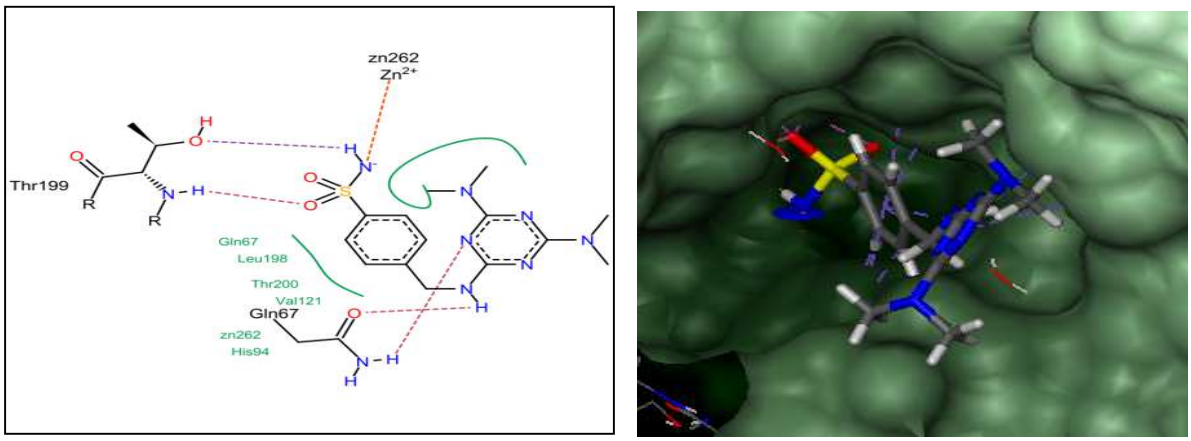


Figure 6: 2D and 3D pose views of compound 24 docked on the active site of hCA IX

The third group of inhibitors with two phenoxy groups on triazine ring; compounds (32-34, figure 1) were weaker inhibitors with K_I values, (254-113 nM).The bulky phenoxy group attached to triazine ring resulted in improper orientation of the sulfonamide group in the active site and interfered with hydrophobic interaction with Leu189,Val121, His49. No water molecules were observed or bind to any compound, as shown in Figure7.

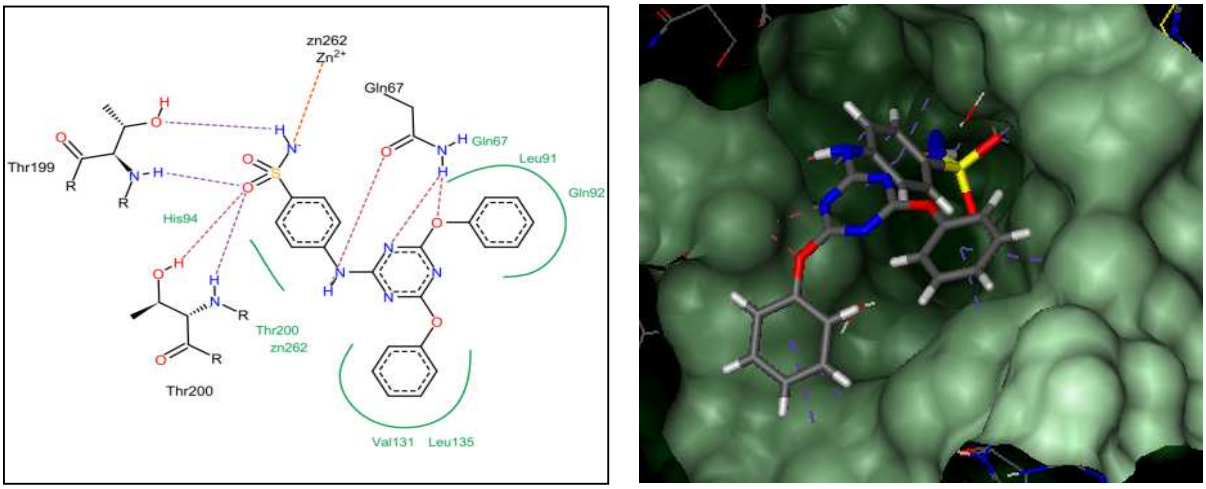


Figure7: 2D and 3D pose views of compound 32 docked on the active site of hCA IX

Compound (32) showed H-bonds formation between sulfonamide group and Thr199 and Thr200. The nitrogen was coordinated to Zn^{2+} ion while benzene ring was sterically hindered with bulky phenoxy groups for any hydrophobic interactions with hydrophobic amino acids in the active site which was reflected negatively on potency. The amino triazine ring formed H-bonds with Gln67 as shown in Figure 7.

The fourth group of compounds (35-38) having amino acid side chain, with $K_I$values, (1-1.7 nM), is highly potent inhibitors. As observed α- or β-amino acid side chains enhanced the potency of these inhibitors. Docked compounds formed H-bonds with Thr199 and Gln67 as well as water molecules (water1412 orwater1489). The carbonyl group of that amino acid substituent formed H-bond with NH of indole ring of the amino acid Trp5 located in active site with inhibitors 35, 36, and 37 as shown in Figure 8.

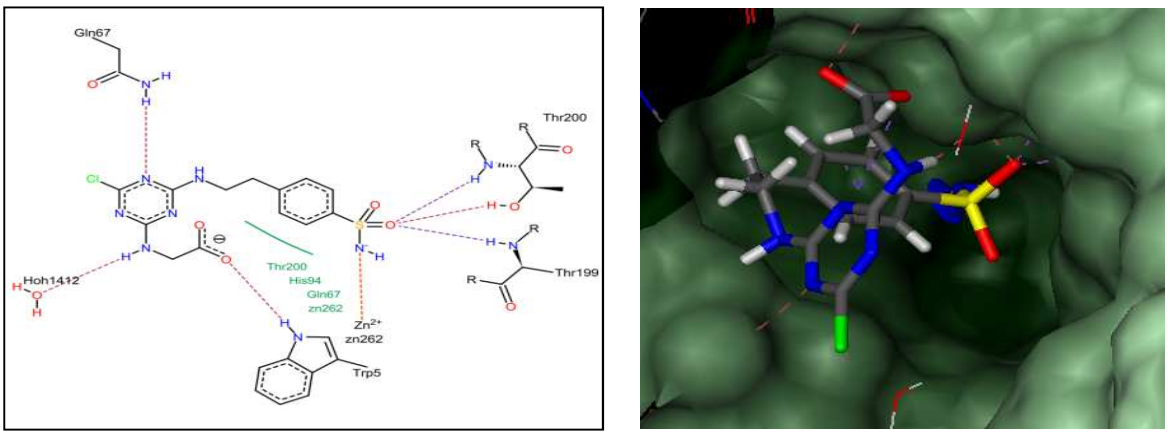


Figure8.2D and 3D pose views of compound 36 docked on the active site of hCA IX

The fifth group of inhibitors, compounds (39-45), is sulfonamide drugs with K_I range (24-52 nM) and they lack the aminotriazinyl group as shown in Figure (1). They all formed H-bond with Thr199 and Gln92. The amino group is coordinated to Zn^{2+} ion in the active site. They did not interact with Gln67 due to absence of aminotriazinyl group. They formed H-bond with either water1214 or water1289 impeded in the active site. The most active members, compounds 39 and 44, had van der Waals interactions with His94, Val121 and Leu198as shown in Figure 9.

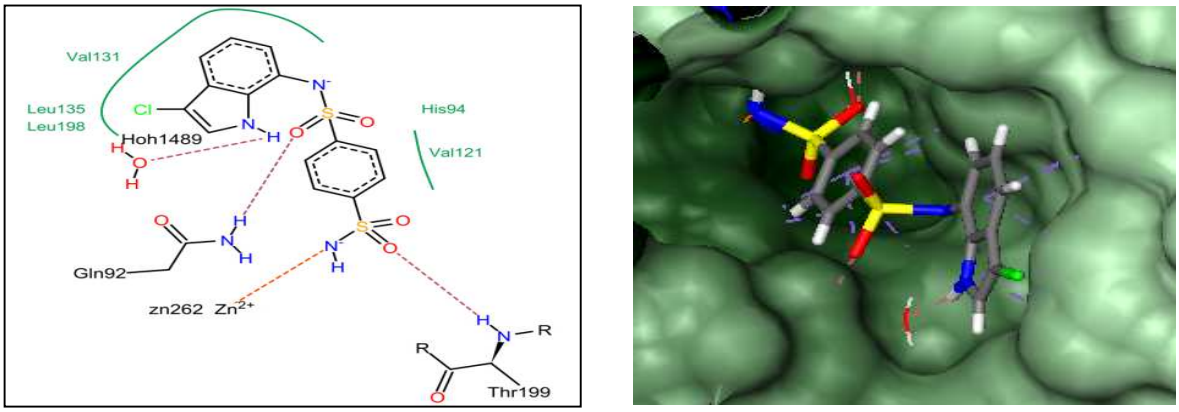


Figure 9.2D and 3D pose views of compound 44 docked on the active site of hCA IX

Concerning compound (44), IND (K_I= 24nM), the oxygen atom of sulfonamide moiety formed H-bond with Thr199 while the nitrogen coordinated to Zn^{2+}ion. The indole ring formed H-bond with water1489.Van der Waals interactions were observed with His94, Val121, Val113, leu135 and Leu198.

CONCLUSION

A new QSAR model with high predictive ability is developed and can be used in the future for modeling and estimating inhibition of hCA IX as a target to design new selective cytotoxic gents.

Molecular docking study of 45heterocyclic sulfonamides as inhibitors of CA IX allowed us to understand the inhibition mechanism. It exploits different interactions with amino acid residues and water molecules from the CA IX active site which offering the possibility to design CAIs with an interesting inhibition profile. QSAR model obtained was supported by the results of docking study. The present QSAR and docking studies suggested that sulfonamide group is essential for activity. The presence of heterocyclic triazine ring enhanced the inhibitory activity. Increasing the size and branching of substituents on the triazine ring reduce the activity. Inhibitors having either free amino, hydrazine or amino acid substituent on triazinyl-sulfonamides were the most potent CA IX inhibitors and cytotoxic agents.

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